ABSTRACT

Nephrotic syndrome is a kidney disease caused by injury of the podocytes. It can be secondary due to infection, systemic disease or certain drugs, but it may also present as sudden primary nephrotic syndrome without obvious inducer. Current standard treatment has many severe adverse effects. In some patients that are resistant to the initial several-week-long glucocorticoid treatment it is possible to reveal the causative genetic aetiology of the disease, whereas in the rest of them aetiology remains unknown. Those who respond well to initial glucocorticoid treatment and achieve remission may later on develop repeated relapses requiring long-term glucocorticoid therapy. This work describes our original research studies focusing on the improvement of genetic diagnostics of nephrotic syndrome, on the exploration of molecular mechanisms of the second most common genetic cause of the steroid-resistant nephrotic syndrome (transcription factor WT1 mutants) and on the search of clinical and laboratory factors that could predict the resistence to glucocorticoid treatment.

By combining Sanger and next-generation sequencing (NGS) we were the first to identify monogenic cause in 38 % of Czech and Slovak children with steroid-resistant nephrotic syndrome whose samples had been collected for 18 years. The most prevalent causative variants were in genes *NPHS2* (15 %), *WT1* (9,5 %) and *NUP93* (5,4 %). A functional study revealed that in 6/8 mutant forms of WT1 transcription factor the DNA-binding affinity was significantly changed. Subsequent luciferase assay in HEK293 cells showed that two WT1 mutant forms presenting with the largest change of the DNA-binding affinity induced the expression of one of the target genes *ACTN1*. The available clinical and laboratory data of the patients did not prove to be predictive of the response to the initial glucocorticoid treatment. The NGS significantly accelerates and reduces the costs of the genetic diagnostics of the monogenic causes of steroid-resistant nephrotic syndrome. The result of the genetic analysis already now allows for effective individualized treatment in some patients. However, in the majority of them including those with know monogenic cause of the disease, we are still waiting for clarification of the the molecular mechanism of the disease. This is an important prerequisite for successful, effective, targeted and side-effects-free treatment, which is nowadays unavailable to most patients.

Key words: functional study, genetic analysis, glucocorticoids, kidney, luciferase assay, mutagenesis, NGS, nephrotic syndrome, binding affinity, WT1